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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING AN IMMUNE RESPONSE

(57) Abstract

There is disclosed a method of stimulating an antigen-specific humoral immune response. Useful vaccine compositions are also disclosed.

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TITLE

METHODS AND COMPOSITIONS FOR MODULATING AN IMMUNE RESPONSE

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of mammalian proteins having immunoregulatory activity, and more specifically to mammalian proteins involved in regulation of a humoral immune response.

BACKGROUND OF THE INVENTION

CD83 is a member of the immunoglobulin superfamily that is expressed on the surface of certain dendritic lineage cells and some lymphoblastoid cell types (Zhou et al., *J. Immunol:* 149:735, 1992; Zhou et al., *J. Immunol.* 154:3821, 1995). The presence of CD83 on dendritic cells has led to the hypothesis that it is somehow involved in antigen presentation; however, prior to the present invention, no biological functions were known for CD83.

Vaccination is an efficient means of preventing death or disability from infectious diseases. Despite the successes achieved with the use of vaccines, however, there are still many challenges in the field of vaccine development. Parenteral routes of administration, the numbers of different vaccinations required and the need for, and frequency of, booster immunizations all impede efforts to control or eliminate disease. Moreover, inability to modulate the type of response, and isotype of antibody made, during immunization has hampered vaccination programs. Although numerous vaccine adjuvants are known, alum is the only adjuvant widely used in humans.

Thus, prior to the present invention, there was a need in the art to determine the function of CD83. There was furthermore a need to develop agents useful in stimulating secretion of antibody, to develop effective methods of immunization, and to discover alternative types of adjuvants, suitable for use in humans.

SUMMARY OF THE INVENTION

The present invention provides a method of stimulating a humoral immune response, comprising administering a CD83 reagent and an antigen, in a pharmaceutically acceptable carrier, wherein the CD83 stimulates production of antigen-specific antibodies Useful CD83 reagents include DNA's encoding CD83 and CD83 polypeptides, as well as derivatives and analogs of such reagents that have CD83 biological activity. The present

invention further provides vaccine compositions useful in stimulating a humoral immune response.

CD83 DNA's that are useful in the inventive methods and compositions include a DNA having a nucleotide sequence encoding an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2 and DNA molecules capable of hybridization to such DNA under stringent conditions and which encode biologically active CD83. Useful CD83 proteins include a CD83 peptide having an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2, fragments of such a peptide according that have CD83 biological activity; and peptides encoded by DNA molecules capable of hybridization to a DNA encoding such peptide under stringent conditions, and which encode biologically active CD83. In a preferred embodiment, CD83 reagents are DNA's that encode, and CD83 peptides that comprise, the extracellular domain of CD83.

Another aspect of the inventive methods and compositions involves administering a cytokine that modulates an immune response in conjunction with a CD43 composition (either sequentially, simultaneously or separately), particularly cytokines selected from the group consisting of Interleukins 1, 2, 4, 5, 6, 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a fusion protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon-γ; TNF; TGF-ß; flt-3 ligand; soluble CD40 ligand; biologically active derivatives of these cytokines; and combinations thereof.

In studies performed using antibodies to CD83, the antibodies inhibited various antigen specific responses. The present invention thus also provides a method of inhibiting undesirable antigen specific responses in a mammal. Such methods of inhibiting undesirable antigen specific responses are useful in preventing or treating autoimmune disease as well as tissue or organ transplant rejection, and in treatment or prevention of allergy or asthma.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates that mice immunized with antigen (DNA encoding TNFr/Fc) in the presence of CD83 have significantly higher serum titers of TNFr/Fc-specific IgG_{2b} than mice immunized with antigen alone.

Figure 2 illustrates the ability of CD83 to stimulate higher levels of antigen-specific IgG_{2b} when the antigen used is a soluble protein antigen (TNFr/Fc).

DETAILED DESCRIPTION OF THE INVENTION

CD83 was cloned from a Raji cell library by polymerase chain reaction, using primers based on the published sequence (Zhou et al., *J. Immunol.* 149:735, 1992). Several different soluble forms of CD83 were expressed, including a Type I Fc/CD83

fusion protein, a Flag®/CD83 fusion protein, and a soluble form of CD83 consisting of the extracellular domain. A detailed explanation of the experimental results and their relevance to the instant invention, along with certain technical background information, are given below.

CD83/HB15

CD83 (also referred to as HB15) is a 45KD glycoprotein predominantly expressed on the surface of dendritic lineage cells, such as skin Langerhans cells and interdigitating reticulum cells present in the T cell zones of lymphoid organs. It is also weakly expressed by some lymphoblastoid cell types, and can be upregulated under certain activation conditions. Structural analysis of the predicted amino acid sequence of this protein established it as a member of the immunoglobulin superfamily (Zhou et al., *J. Immunol.* 149:735, 1992). It has more recently been shown that human blood dendritic cells express CD83 (Zhou et al., *J. Immunol.* 154:3821, 1995). U.S. Patent 5,316,920, issued May 31, 1994, discloses and claims DNA's encoding CD83; WO93/21318 is a corresponding published international patent application. WO 95/29236 discloses related proteins and DNA's encoding them as well as antibodies reactive with these proteins.

Although the presence of CD83 on dendritic cells has led to the hypothesis that it is somehow involved in antigen presentation, prior to the present invention, no biological functions were known for this protein. The discovery that CD83 stimulates production of antibodies led to the inventive uses and compositions described herein. Because of its role, CD83 (both in protein form and in DNA form) will be useful as a vaccine adjuvant. CD83 can be administered in conjunction with other immunomodulatory molecules, as described herein. Moreover, DNA ending CD83 can be incorporated into attenuated live viral or bacterial vaccine strains, to enhance the immune response to the infectious agent. Additionally, antagonists of CD83 will be useful in suppressing an undesirable, antigen-specific immune response.

The protective immune response

An immune response to a pathogen can be classified broadly as either being cell-mediated (cellular immunity) or antibody mediated (humoral immunity). In cellular immunity, activated macrophages and cytotoxic lymphocytes carry out elimination of the pathogen. Humoral immunity, in contrast, operates primarily through antibody production. It is currently believed that these two arms of the immune response are regulated by distinct subsets of helper T (T_H) cells which secrete specific arrays of cytokines (reviewed in *Immunological Reviews* 123, 1991).

Type 1 T_H cells (T_H1 cells) mediate delayed type hypersensitivity (DTH), and secrete Interferon-γ (IFN-γ) and Interleukin-2 (IL-2), while Type 2 T_H cells (T_H2 cells)

secrete primarily Interleukins 4, 5 and 10 (IL-4, IL-5 and IL-10, respectively) and provide B cell help. Development of the immune response along either T_H1 or T_H2 pathway is often apparent early in an infection, and appears to be governed by the type of organism causing the infection (Scott and Kaufmann, *Immunol. Today* 12:346, 1991), and by the genetic makeup of the infected host. Failure to resolve disease or development of immunopathology can result when the immune response proceeds inappropriately.

The immune response may be manipulated toward either a T_H1 or T_H2 by the appropriate administration of cytokines, or cytokine antagonists. For example, administration of IFN-γ or an antibody that neutralizes IL-4 would enhance a T_H1 response, whereas administration of IL-10 or a molecule that inhibited the action of IFN-γ would stimulate a T_H2 response. This ability to manipulate the immune response provides a useful tool not only in infectious disease, but in inflammatory and allergic diseases as well (see, for example, Powrie and Coffman, *Immunol. Today* 14:270, 1993).

Early antibody responses, both in the life cycle of an animal and in the ontogeny of individual B cell clones, primarily consist of IgM. Under the control of helper T cells, the isotype of antibody produced by B cells is switched from IgM to IgG, IgE or IgA. The latter isotypes are representative of a more mature immune response, and generally include antibodies of higher affinity and avidity as well as increased effector function. Stimulation of non-IgM isotypes is considered a desirable effect of any vaccination protocol, since it is the IgG, IgE and IgA antibodies that are generally protective against infectious disease, and which are likely to play a role in tumor immunity. Moreover, the IgG subclasses are preferred for the generation of monoclonal antibodies, since these exhibit useful characteristics (i.e., easier purification, higher affinity, greater therapeutic effectiveness due to enhanced effector functions).

Vaccines and disease

Immunization is a centuries old, and highly effective, means of inducing a protective immune response against pathogens in order to prevent or ameliorate disease. The vaccines that have been used for such induction are generally live, attenuated microorganisms, or preparations of killed organisms or fractions thereof. Live, attenuated vaccines are generally thought to more closely mimic the immune response that occurs with a natural infection than do those prepared from killed microbes or non-infective preparations derived from pathogens (i.e., toxoids, recombinant protein vaccines). However, attenuated vaccines also present a risk of reversion to pathogenicity, and can cause illness, especially in immunocompromised individuals.

Along with improved sanitation, immunization has been the most efficient means of preventing death or disability from numerous infectious diseases in humans and in other animals. Vaccination of susceptible populations has been responsible for eliminating small

pox world wide, and for drastic decreases in the occurrence of such diseases as diphtheria, pertussis, and paralytic polio in the developed nations. Numerous vaccines are licensed for administration to humans, including live virus vaccines for certain adenoviruses, measles, mumps and rubella viruses, and poliovirus, diphtheria and tetanus toxoid vaccines, and *Haemophilus* b and meningococcal polysaccharide vaccines (Hinman et al., in Principles and Practice of Infectious Diseases, 3rd edition; G.L. Mandell, R.G. Douglas and J.E. Bennett, eds., Churchill Livingstone Inc., NY, NY; 2320-2333; Table 2).

Despite the successes achieved with these vaccines, however, there are still numerous challenges in the field (*Science* 265:1371; 1994). HIV infection is a public health problem in both developed and developing nations; there has been little progress in developing an effective vaccine against this virus despite significant research efforts in this area. Malaria and tuberculosis represent significant public health challenges in the developing world, with high morbidity and mortality rates, and problematic treatment regimes. Respiratory syncytial virus (RSV) and pneumococcal disease pose similar difficulties in the developed world.

Even for diseases for which there are effective vaccines available, maintaining an sufficient rate of immunization in susceptible populations presents a public health challenge. Many children in the United States are not vaccinated for common childhood diseases such as diphtheria and pertussis. Adults may not receive necessary boosting immunizations for tetanus or other diseases. Parenteral routes of administration, the numbers of different vaccinations required and the need for, and frequency of, booster immunizations all impede efforts to achieve patient compliance with vaccine programs. Developing countries also face additional challenges in trying to store and administer vaccines.

Several aspects of vaccine preparation and administration have been investigated. These include route of administration and encapsulation of antigen preparations to provide sustained release of the antigen (see, for example, USSN 08/508,229, filed July 27, 1995), and the use of adjuvants. Useful adjuvants include for example alum, fragments of bacterial membranes, liposomes, coupling a protein of interest to a larger immunogenic protein, RIBI, non-ionic block co-polymer surfactants and TiterMax®. Other useful vaccine adjuvants and excipients are described by Vogel and Powell (A Compendium of Vaccine Adjuvants and Excipients, in: Vaccine Design, Powell and Newman, eds.; Plenum Publishing Corporation, NY, NY; 1994). Of these, alum is the only adjuvant widely used in humans.

Other areas of interest in the field of vaccination are the use of cytokines to modulate an immune response. Some cytokines, e.g., interleukin-4 (IL-4) and GM-CSF, attract and activate antigen-presenting cells for more efficient presentation of antigens to T cells. These cytokines have been co-administered with antigen to increase antigenic

activity. Other studies have shown that the host response to tumor challenge can be increased by inoculation of tumor cells genetically engineered to express particular cytokines, including γ-IFN, TNF-α, IL-2, IL-4, IL-6, IL-7, or GM-CSF. Recombinant antigens have been expressed as fusion proteins with cytokines, for example as described in USSN 08/271,875, filed July 7, 1994.

The use of "naked DNA" represents one of the newest approaches to vaccination (Pardoll and Beckerleg. *Immunity* 3:165, 1995). The utility of DNA in vaccine preparations rests upon the ability of purified DNA to be taken up and expressed by cells in vivo with much greater efficiency than is seen in vitro. Large scale production of DNA is relatively simple, and the resulting DNA can be readily purified to a very great degree, reducing the potential for dangerous contaminants. Moreover, purified DNA is much more stable than purified proteins and other biological materials, which can ameliorate storage and administration problems.

DNA's, Proteins and Analogs

The present invention provides isolated CD83 DNA's and proteins (referred to as CD83 agents) having immunoregulatory activity. Such DNA's and proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of the CD83 proteins within the scope of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a CD83 protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Other derivatives of the CD83 protein within the scope of this invention include covalent or aggregative conjugates of the protein or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α-factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of CD83 proteins (e.g., poly-His). The amino acid sequence of the CD83 proteins can also be linked to an identification peptide such as that described by Hopp et

al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein.. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

Fusion proteins further comprise the amino acid sequence of a CD83 protein linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG1 having a nucleotide and amino acid sequence set forth in SEQ ID NO:3. Fragments of an Fc region may also be used. Depending on the portion of the Fc region used, a CD83 protein may be expressed as a dimer, through formation of interchain disulfide bonds. If CD83 fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four CD83 protein regions.

In another embodiment, CD83 proteins further comprise an oligomerizing zipper domain. Oligomerizing zipper domains are described in USSN 08/107,353, filed August 13, 1993, the relevant disclosure of which is incorporated by reference herein. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989), the nuclear transforming proteins, *fos* and *jun*, which preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989), and the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990).

Other useful fusion proteins include fusions of CD83 with an antigen against which it is desired to elicit an immune response, for example as described in USSN 08/271,875, filed July 7, 1994, for GM-CSF. Similarly, fusion proteins consisting of CD83 and another cytokine or cytokines are also contemplated. As shown herein for CD83 DNA's, the DNA's encoding such fusion proteins will also have utility in the instant invention. A very useful DNA may include not only sequences encoding CD83 and another cytokine (for example, CD40L), but also sequences encoding the antigen(s).

CD83 protein derivatives may also be used as immunogens, reagents in immunoassays, or as binding agents for affinity purification procedures, for example, in purifying CD83 antibodies. CD83 protein derivatives may also be obtained by cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. CD83 proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated,

bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the CD83 protein or against other proteins which are similar to the CD83 protein.

The present invention also includes CD83 proteins with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of CD83 DNA's in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of CD83 protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A$_1$-Z, where A$_1$ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A$_1$ and Z, or an amino acid other than Asn between Asn and A$_1$.

CD83 protein derivatives may also be obtained by mutations of the DNA encoding native CD83 protein or its subunits. An CD83 mutated protein, as referred to herein, is a polypeptide homologous to a CD83 protein but which has an amino acid sequence different from the native CD83 protein because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a CD83 peptide may be easily determined by analyzing the ability of the mutated CD83 peptide to bind antibodies to CD83, or by analyzing the ability of the CD83 mutein to stimulate secretion of antibody classes characteristic of a secondary immune response as described herein

Bioequivalent analogs of CD83 proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the ability of the CD83 proteins to bind CD83 antibodies, or to stimulate secretion of antibodies from human cells. Examples of

conservative substitutions include substitution of amino acids outside of the binding domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of CD83. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Subunits of CD83 proteins may be constructed by deleting terminal or internal residues or sequences. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of CD83 to the sequences and structures of other immunoglobulin superfamily members.

Mutations in nucleotide sequences constructed for expression of analog CD83 proteins must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed mutated CD83 proteins screened for the desired activity.

DNA's that encode any of the forgoing CD83 peptides will also be useful in stimulating a humoral immune response, as will other CD83 DNA's. For example, not all mutations in the nucleotide sequence which encodes a CD83 protein will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene*

37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent Nos. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Such degenerate CD83 DNA's will also be useful in the instant invention. Other embodiments include DNA's capable of hybridizing under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding CD83 protein. Conditions of higher stringency are known in the art; DNA's hybridizing under stringent conditions represent a preferred embodiment. In a preferred embodiment, CD83 peptides are at least about 70% identical in amino acid sequence to the amino acid sequence of CD83 as set forth in SEQ ID NO:1. In a most preferred embodiment, analogs of CD83 proteins are at least about 80% identical in amino acid sequence to the native form of the proteins.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For fragments derived from the CD83 protein, the identity is calculated based on that portion of the CD83 protein that is present in the fragment.

Purification of CD83 proteins or DNA's

Purified CD83 proteins or analogs are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNA's described herein, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a CD83 antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying CD83 proteins.

Affinity chromatography is a particularly preferred method of purifying CD83 proteins. For example, a CD83 protein expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a CD83 protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against a CD83 protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a viral protein composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant CD83 protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant CD83 protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express CD83 protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

CD83 protein synthesized in recombinant culture is characterized by the presence of non-CD83 cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the viral protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of CD83 protein free of other proteins which may be normally associated with the CD83 protein as it is found in nature in its species of origin.

Useful CD83 DNA's may be purified by any suitable method of purifying DNA's known in the art. Several useful methods are described in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York, second edition; 1989), particularly in Chapter 1, in the section relating to extraction and purification of plasmid DNA (1.21). For example, DNA is amplified in prokaryotic

cells, and isolated by a standard alkaline lysis procedure followed by resin purification as described in standard kits (for example, from Promega Biotec, Madison, WI, or Quiagen, Chatsworth, CA). The isolated DNA is then resuspended in a suitable diluent or carrier.

Administration of CD83 Protein and DNA Compositions

The present invention provides methods of using therapeutic compositions comprising an effective amount of a CD83 reagent and a suitable diluent and carrier, and methods for regulating an immune response. The use of CD83 proteins in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated. CD83 DNA's and/or proteins are administered for the purpose of stimulating a humoral immune response.

For therapeutic use, a purified CD83 reagent is administered to an individual, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, CD83 protein compositions administered to stimulate a humoral immune response can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified CD83 protein in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such CD83 protein compositions entails combining the CD83 protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the individual, the nature of an antigen for which the CD83 is being used as an adjuvant, and so forth.

Since DNA can be integrated into the genome, or be maintained in episomal form, DNA vaccines provide the potential for long-term expression of antigens, with commensurate duration of an immune response. Simple saline solutions appear to be suitable carriers for DNA vaccines, and various routes of administration have been shown to be useful, including intramuscular (Ulmer et al., *Science* 259:1745, 1993; Montgomery et al., *DNA Cell Biol.* 12:777, 1993) or intradermal injection (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994), as well as the use of a "gene gun" (Tang et al., *Nature*

365:152, 1992; Fynan et al., *Proc. Natl. Acad. Sci. USA* 90:1148, 1993; Eisenbraun et al., *DNA Cell Biol.* 12:791, 1993).

For use is stimulating a certain type of immune response, administration of other cytokines along with either CD83 DNA or CD83 protein, is also contemplated. Several useful cytokines (or peptide regulatory factors) are discussed in Schrader, J.W. (*Mol Immunol* 28:295; 1991). Such factors include (alone or in combination) Interleukins 1, 2, 4, 5, 6, 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a fusion protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon-γ, TNF, TGF-ß, flt-3 ligand and biologically active derivatives thereof. A particularly preferred cytokine is CD40 ligand (CD40L). A soluble form of CD40L is described in USSN 08/484,624, filed June 7, 1995. DNA encoding such cytokines will also be useful in the inventive methods. Administration of these immunomodulatory molecules includes simultaneous, separate or sequential administration with suitable CD83 compositions (proteins or DNA's) and antigens.

Antagonists of CD83 will be useful in inhibiting a humoral immune response. Exemplary conditions in which it is advantageous to inhibit such undesirable responses include autoimmune syndromes, including myasthenia gravis, multiple sclerosis and systemic lupus erythematosis, and others as described in U.S. Patent 5,284,935. Moreover, CD83 antagonists can also be useful to prevent or treat rejection of tissue and/or organ transplants. Other conditions for which CD83 antagonists can be useful include those which involve undesirable immune responses to foreign antigens, for example those which occur in allergy or asthma.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

This example describes construction of an HB15 (CD83) DNA construct to express a soluble CD83 protein. Using conventional techniques of PCR amplification, enzyme cutting and ligation, several CD83-encoding constructs were prepared, including one encoding a CD83/Fc fusion protein, a Flag®/CD83 protein, and a soluble form of CD83. An expression vector (pDC409, which differs from pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991) in that a *Bgl* II restriction site outside of the multiple cloning site has been deleted, making the *Bgl* II site within the multiple cloning site unique) comprising appropriate regulatory elements, and sequences encoding the signal peptide and

extracellular domain of CD83 from amino acid -19 to amino acid 124 of SEQ ID NO:1, along with a suitable Fc region of an immunoglobulin (SEQ ID NO:3; three amino acids in the hinge region have been changed to reduce affinity for Fc receptor) was prepared and expressed. The resulting fusion protein was referred to as CD83/Fc Type I.

A soluble form of CD83 from amino acid -19 to amino acid 124 of SEQ ID NO:1, and a Flag® form consisting of amino acid -19 to amino acid 124 of SEQ ID NO:1 linked to the eight amino acid sequence described by Hopp et al. (*Bio/Technology* 6:1204,1988; SEQ ID NO:5) were prepared in an expression vector (pDC304, which is derived from pDC302, described by Mosley et al., *Cell*, 59:335 (1989), by deleting the adenovirus tripartite leader (TPL) in pDC302).

The resultant expression vectors are transfected into the monkey kidney cell line CV-1/EBNA (ATCC CRL 10478). Large scale cultures of CV-1/EBNA cells transfected with the various constructs are grown to accumulate supernatant containing the different forms of CD83 protein. The CV-1/EBNA cell line permits expression of recombinant proteins ligated into vectors containing the EBV origin of replication. The CD83 proteins in supernatant fluid are purified as described below.

EXAMPLE 2

This example illustrates purification of various forms of CD83 proteins. The Flag®/CD83 protein was purified by affinity chromatography. Briefly, culture supernatant containing the Flag®/CD83 protein was purified by filtering mammalian cell supernatants (e.g., in a 0.45μ filter) and applying filtrate to an affinity column comprising a monoclonal antibody specific for the Flag® peptide, coupled to Affi-gel active ester agarose (Bio-Rad, Richmond, CA), at room temperature, at a flow rate of approximately 60 to 80 ml/hr for a 1.5 cm x 12.0 cm column. The column was washed with approximately 20 column volumes of PBS (phosphate buffered saline), until free protein could not be detected in wash buffer. Bound CD83 protein was eluted from the column by competition with excess Flag® peptide (100 μg/ml in PBS), and stabilized in 10% glycerol.

CD83/Fc protein is purified by conventional methods using Protein A or Protein G chromatography. Approximately one liter of culture supernatant containing CD83 protein is purified by filtering mammalian cell supernatants (e.g., in a 0.45m filter) and applying filtrate to a protein A/G antibody affinity column (Schleicher and Schuell, Keene, NH) at 4°C at a flow rate of 80 ml/hr for a 1.5 cm x 12.0 cm column. The column is washed with 0.5 M NaCl in PBS until free protein is not detected in the wash buffer. Finally, the column is washed with PBS. Bound fusion protein is eluted from the column with 25 mM citrate buffer, pH 2.8, and brought to pH 7 with 500 mM Hepes buffer, pH 9.1.

Additional constructs can be prepared and the expressed protein purified using methods that are known in the art. For example, a CD83 protein comprising a poly-His

peptide may be detected and/or purified using a poly-His system, substantially as described in US Patent 5,284,933, issued February 8, 1994.

Ability to bind antibodies to CD83 is used as an assay for detection of CD83 activity. Biological activity is measured in any biological assay which quantifies an antigen-specific immune response, for example, as described in the Examples herein.

EXAMPLE 3

This example illustrates the effect of CD83/Fc on a secondary immune response. On day 0, 6 BALB/c mice were injected subcutaneously with 200µl of a suspension containing 4 µg of ovalbumin (OVA). On day 14, they were injected with either 500 µg of CD83/Fc or 500 rat IgG as a control. Six hours later they were re-immunized with 1 µg ovalbumin. The mice were bled at day 21 and titers of ovalbumin-specific antibodies of various subclasses were determined by ELISA. The mice that were given CD83/Fc prior to the secondary immunization exhibited slightly higher levels of certain subclasses of ovalbumin-specific antibodies, particularly IgG_{2b}.

EXAMPLE 4

The example illustrates the ability of DNA encoding CD83 to stimulate a primary humoral immune response with high levels of IgG_{2b}. BALB/c mice (six per group) were injected with 50 µg of DNA comprising DNA encoding a tumor necrosis factor/immunoglobulin Fc fusion protein (described by Mohler et al., *J Immunol* 151:1548,1993; and in EP 418014), along with either 50 µg CD83-encoding vector DNA or control vector DNA. Mice were bled at day 14 for determination of TNFr/Fc-specific antibody titers by ELISA. Results are shown in table 1 below; titration curves for the TNFr/Fc-specific IgG_{2b} are shown in Figure 1. The results demonstrated that CD83 cDNA significantly enhanced antigen-specific antibody titers of all isotypes, especially IgG_{2a} and IgG_{2b}.

Table 1: End-point titers of TNFr/Fc-specific antibody in animals injected with TNFr/Fc DNA and either control DNA or CD83 DNA*

	Control DNA	CD83 DNA
IgG1	218,700	1,968,300
IgG2a	900	72,900
IgG2b	2700	218,700
IgA	2700	72,900
IgE	300	2700

* Lowest reciprocal dilution of sera at which all animals in each group have detectable titers.

In a second experiment, mice were injected with either 50 µg CD83-encoding vector DNA (6 mice) or control vector DNA (5 mice) at day 0, given intradermally near the base of the tail. At day 3, they were given 5 µg of TNFr/Fc protein subcutaneously at the back of the neck. The mice were bled at day 14 for determination of TNFr/Fc-specific antibody titers by ELISA. Results are shown in table 2 below; titration curves for the TNFr-specific IgG_{2b} are shown in Figure 2. The results confirmed that CD83 cDNA significantly enhanced antigen-specific antibody titers of all isotypes, especially IgG_{2a} and IgG_{2b}.

Table 2: End-point titers of TNFr/Fc-specific antibody in animals injected with TNFr/Fc protein and either control DNA or CD83 DNA*

	Control DNA	CD83 DNA
IgG1	72,900	656,100
IgG2a	900	72,900
IgG2b	2700	218,700
IgA	2700	24,300
IgE	100	2700

* Lowest reciprocal dilution of sera at which all animals in each group have detectable titers.

These results demonstrated that use of CD83 as a vaccine adjuvant results in a primary humoral immune response that qualitatively and quantitatively resembles a secondary immune response.

EXAMPLE 5

This example illustrates the ability of CD83 proteins to induce isotype switching in naive human B cells. Naive B cells are obtained by one of several methods known in the art. For Example, surface IgD-positive tonsillar B cells, neonatal B cells obtained from cord blood and B cell obtained from individuals suffering from X-linked hyper-IgM syndrome represent populations of cells that are substantially devoid of isotype-committed B cells. Mononuclear cells are isolated by a method such as centrifugation over Ficoll-Hypaque, and depleted of T cells by rosetting with 2-aminoethylisothiouronium bromide-treated SRBC (sheep red blood cells). The resulting E- cells can be further purified by negative selection using antibodies to cell surfaces markers found on non-B cells (i.e., CD2, CD3, CD14), and/or by positive selection using antibodies to markers found on non-isotype committed B cells (i.e., sIgD). The cells are cultured under suitable conditions, and stimulated with cytokines to induce immunoglobulin secretion. The cytokines to be used include a soluble trimeric from of CD40 ligand (CD40L) as described in USSN 08/484,624, filed June 7, 1995; IL-4, and IL-10. Other cytokines may also be included, for example, transforming growth factor ß and IL-2. After stimulation, supernatants are harvested and tested for the presence of various classes of immunoglobulins by ELISA. CD83 stimulates secretion of IgG_{2b} from murine B cells under such conditions; it will likewise stimulate isotype switching and secretion of IgG_2 from naive human B cells.

EXAMPLE 6

This example illustrates the preparation of monoclonal antibodies against CD83. Preparations of purified recombinant CD83/Fc, for example, or transfected cells expressing high levels of CD83, are employed to generate monoclonal antibodies against CD83 using conventional techniques such as those disclosed in U.S. Patent 4,411,993. Such antibodies are likely to be useful in interfering with CD83 binding or biological activity, as components of diagnostic or research assays for CD83, or in affinity purification of CD83.

To immunize rodents, CD83 immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich) or ELISA (enzyme-linked immunosorbent assay). Other assay procedures are also suitable, for example, FACS analysis using cells expressing membrane-bound CD83. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in

saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with CD83, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Patent 4,703,004. A preferred screening technique utilizes fluorescence activated cell sorting to detect binding to cells that express CD83, for example, Raji cells. Positive clones are then generated and injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-CD83 monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to CD83 protein.

Using these methods, three hybridoma clones secreting antibodies that bound CD83 were generated; the antibodies were referred to as M43, M240, and M245. The antibodies were able to partially compete with each other for CD83 binding, as determined by ELISA and FACS; initial results indicated that they bound to slightly different epitopes. The antibodies were also able to inhibit antigen-specific proliferation of peripheral blood T cells.

EXAMPLE 7

This example describes two solid-phase binding assays, the first of which, (a), can be used to quantify soluble CD83, and the second of which, (b), is used to detect the presence of soluble CD83.

(a) Quantitative CD83 ELISA

Antibody to CD83 is purified and used to coat 96-well plates (for example, Corning EasyWash ELISA plates, Corning, NY, USA). In a preferred method, the plates are coated for one hour at room temperature with 50 µl of PBS containing 5 µg/ml of M43 (described in Example 6), and blocked with 100 µl/well of 5 % non-fat dried milk in PBS for 1 hour at room temperature. Samples to be tested are diluted in 10% normal goat serum (NGS) in PBS, and 50 µl is added per well. A titration of unknown samples is run in duplicate, and a titration of reference standard of CD83 is run to generate a standard curve. The plates are incubated with the samples and controls for one hour at room temperature, then washed four times with PBS. Second step reagent, for example, rabbit anti-CD83 (50 µl/well, diluted 1:500 in PBS/10 % NGS), is added and the plates are incubated at room

temperature for one hour. The plates are again washed as previously described, and donkey anti-rabbit IgG conjugated to horseradish peroxidase (Jackson Labs Westgrove, PA; diluted 1:2000 in PBS/10 % NGS) is added. Plates are incubated for one hour at room temperature, washed as described, and the presence of CD83 is detected by the addition of chromogen/substrate, tetramethyl benzidene/peroxidase (TMB, 50 µl/well; Kirkegard and Perry, Gaithersberg, MD) at room temperature until development of color. The chromogenic reaction is stopped by the addition of 50 µl/well 2N H_2SO_4, and the OD_{450} of the wells determined. The quantity of soluble CD83 can be determined by comparing the OD values obtained with the unknown samples to the values generated for the standard curve. Values are expressed as the number of picograms per ml.

(b) Qualitative Dot Blot

Soluble CD83 (1 µl of crude supernatant or column fractions) is adsorbed to dry BA85/21 nitrocellulose membranes (Schleicher and Schuell, Keene, NH) and allowed to dry. The membranes are incubated in tissue culture dishes for one hour in Tris (0.05 M) buffered saline (0.15 M, pH 7.5 containing 1% w/v BSA to block nonspecific binding sites. At the end of this time, the membranes are washed three times in PBS, and rabbit anti-CD83 antibody is added at an approximate concentration of 10 µg/ml in PBS containing 1% BSA, following which the membranes are incubated for one hour at room temperature. The membranes are again washed as described, and a horseradish peroxidase (HRP)-labeled antibody such as goat anti-rabbit Ig; Southern Biotech, Birmingham, AL) at an approximate dilution of 1:1000 in PBS containing 1% BSA is added. After incubating for one hour at room temperature, the membranes are washed and chromogen (i.e. 4-chloronaphthol reagent, Kirkegard and Perry, Gaithersburg, MD) is added. Color is allowed to develop for ten minutes at room temperature, and the reaction is stopped by rinsing the membranes with water. The membranes are washed, and the presence of soluble CD83 is determined by analyzing for the presence of a blue-black color. This assay is used to determine the presence or absence of soluble CD83 in cell culture supernatant fluids and in purification column fractions. The assay further provides a semi-quantitative method of determining relative amounts of soluble CD83 by comparing the intensity of the color in unknown samples to the intensity of known quantities of controls.

EXAMPLE 8

This example demonstrates that soluble CD83 is shed from the surface of activated peripheral blood B and T cells. Peripheral blood B and T cells are obtained by any suitable method known in the art. For example, PBMCs are isolated from a healthy donor by centrifugation of heparinized blood over Isolymph (Gallard-Schlesinger Industries, Inc., Norway) and washed three times (i.e., with culture medium consisting of RPMI 1640 supplemented with 10% FCS, 50 U/ml penicillin, 50 µg/ml streptomycin, 2mM

glutamine). Isolated PBMCs are separated into T cell and non-T-cell fractions by rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells. Twice rosetted cells are suspended in RPMI 1640 culture media with 10 % FCS and incubated on plastic dishes for 1 hr at 37°C to remove any remaining adherent cells. The resulting cell preparations were always at least 90% T cells (98% $CD2^+$; 90% $CD3^+$) as determined by flow cytometric analysis B cells are further purified from the E^- preparation by positive selection using CD19 monoclonal antibody (mAb) on a MACS column (Miltenyi Biotec, Sunnyvale, CA) according to the manufacturers instructions. $CD19^+$ B cells purified in this way were routinely >95% pure as determined by reactivity with CD20 mAb.

Peripheral blood T or B cells were cultured at $1x10^6$ in 1ml for 48 or 24 hours with the following stimuli: immobilized CD3 mAb, 5µg/ml; PHA, 1%; IL-2, 10ng/ml; PMA, 10ng/ml; Ionomycin, 500ng/ml; IL-4, 10ng/ml; soluble trimeric CD40L, 2µg/ml; SAC, 0.01%. Levels of soluble CD83 in culture supernatants were measured by ELISA A protease inhibitor, TAPI (described in USSN 08/292,547, filed August 18, 1994, now allowed; 100µM final concentration), was included in the culture medium to ascertain whether a proteolytic reaction was required for expression of soluble CD83. After culture for either 48 hours (T cells) or 24 hours (B cells), 50 µl of supernatant fluid was removed and tested for the presence of soluble CD83 by quantitative EIA as described in Example 7 above. Results (expressed in pg/ml of CD83) are shown in Table 3 below.

Table 3: Shedding of Soluble CD83 from Activated Lymphoid Cells

	Stimulus	w/o TAPI	w TAPI
PB T cells 48hr + :	medium	<3	<3
	αCD3	57.6	29.5
	PHA	65.0	<3
	αCD3 + IL-2	53.2	<3
	PHA + IL-2	60.5	<3
	PHA + PMA	154.8	34.6
	PMA + Iono	59.1	<3
PB B cells 24hr + :	medium	94.0	<3
	IL-4	127.1	<3
	CD40L	288.1	48.4
	SAC	375.9	138.7
	CD40L + IL-4	478.7	63.5
	SAC + IL-4	529.2	202.2

These results indicated that soluble CD83 was expressed as a result of a proteolytic cleavage of membrane-bound CD83. In general, B cells produced larger quantities of soluble CD83 than did T cells, and for both cell types, certain stimuli caused shedding of greater quantities of CD83 than did other stimuli.

EXAMPLE 9

This example illustrates the ability of CD83 proteins to enhance immunoglobulin secretion from human B cells. Isotype-switched B cells are obtained by one of several methods known in the art. For example, surface IgD-negative B cells are further purified from peripheral mononuclear cells (isolated by a method such as centrifugation over Ficoll-Hypaque, and depleted of T cells by rosetting with 2-aminoethylisothiouronium bromide-treated SRBC) and depleting the E^- cells of IgD^+ B cells (i.e., by positive selection on a MACS column). The resulting cells can also be further purified by positive selection for a B cell marker such as CD19. IgD^- B cells may also be obtained from tonsils, using methods that are known in the art (for example, Liu et al., *Eur. J. Immmunol.* 21:1107, 1991; or Lagresle et al., *Int. Immunol.* 5:1250, 1993)

IgD^- peripheral blood B cells ($5x10^4$/well) were cultured for twelve days in the presence of soluble, trimeric CD40L (2μg/ml) and IL-2 (10ng/ml); IL-6 (10ng/ml) or IL-10 (20ng/ml) were also included in some cultures. Recombinant soluble CD83 was added to cultures at a final concentration of 10 or 100ng/ml. IgG1 and IgG2 levels in culture supernatants were determined at day twelve by ELISA. The results are shown in Table 4.

Table 4: Effect of Soluble CD83 of Immunoglobulin Secretion by Human B Cells

		Immunoglobulin (ng/ml)		
	Amount sCD83	CD40L + IL-2	CD40L + IL-2 + IL-6	CD40L + IL-2 + IL-10
IgG_1	0 ng/ml	68	280	419
	10 ng/ml	744	4572	1533
	100 ng/ml	553	2080	882
IgG_2	0 ng/ml	<10	<10	<10
	10 ng/ml	183	1360	430
	100 ng/ml	260	1366	440

These results demonstrated that soluble CD83 enhanced secretion of both IgG_1 and IgG_2 by IgD^- human peripheral blood B cells.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: IMMUNEX CORPORATION

(ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR MODULATING AN IMMUNE RESPONSE

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Apple Operating System 7.5.2
(D) SOFTWARE: Microsoft Word for Power Macintosh, Version 6.0.1

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 618 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: HB15

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..618

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..615

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ser Arg Gly Leu Gln Leu Leu Leu Leu Ser Cys Ala Tyr Ser Leu
-19                 -15                 -10                 -5

GCT CCC GCG ACG CCG GAG GTG AAG GTG GCT TGC TCC GAA GAT GTG GAC          96
Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu Asp Val Asp
                 1               5                  10

TTG CCC TGC ACC GCC CCC TGG GAT CCG CAG GTT CCC TAC ACG GTC TCC         144
Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr Thr Val Ser
     15                  20                  25

TGG GTC AAG TTA TTG GAG GGT GGT GAA GAG AGG ATG GAG ACA CCC CAG         192
Trp Val Lys Leu Leu Glu Gly Gly Glu Glu Arg Met Glu Thr Pro Gln
 30                  35                  40                  45

GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GGG CAA AAT GGT         240
Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly Gln Asn Gly
                 50                  55                  60

TCT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG ATC CGA AAC         288
Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys Ile Arg Asn
             65                  70                  75

ACT ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG CAG GAC CCG         336
Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu Gln Asp Pro
         80                  85                  90

GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA GTG ACA GGA         384
Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg Val Thr Gly
     95                 100                 105
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TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC AGA GCG GAG        432
Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr Arg Ala Glu
110                 115                 120                 125

ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA CTC ATC ATT        480
Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr Leu Ile Ile
                130                 135                 140

TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA GAT TTT TCT        528
Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro Asp Phe Ser
            145                 150                 155

AAA GCT GGC ATG GAA AGA GCT TTT CTC CCA GTT ACC TCC CCA AAT AAG        576
Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser Pro Asn Lys
        160                 165                 170

CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA TGA                618
His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  *
    175                 180                 185
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ser Arg Gly Leu Gln Leu Leu Leu Leu Ser Cys Ala Tyr Ser Leu
-19                 -15                 -10                  -5

Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu Asp Val Asp
                  1                   5                  10

Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr Thr Val Ser
      15                  20                  25

Trp Val Lys Leu Leu Glu Gly Gly Glu Glu Arg Met Glu Thr Pro Gln
 30                  35                  40                  45

Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly Gln Asn Gly
                  50                  55                  60

Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys Ile Arg Asn
              65                  70                  75

Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu Gln Asp Pro
          80                  85                  90

Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg Val Thr Gly
      95                  100                 105

Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr Arg Ala Glu
110                 115                 120                 125

Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr Leu Ile Ile
                130                 135                 140
```

```
Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro Asp Phe Ser
            145                 150                 155

Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser Pro Asn Lys
        160                 165                 170

His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val
    175                 180                 185
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 699 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: HuIgG Fc mutein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..699

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GAG CCC AGA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA      48
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
  1               5                  10                  15

CCT GAA GCC GAG GGT GCG CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC      96
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG     144
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG     192
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG     240
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG     288
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
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GAC TGG CTG AAT GGC AAG GAC TAC AAG TGC AAG GTC TCC AAC AAA GCC        336
Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
                100                 105                 110

CTC CCA GCC CCC ATG CAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC        384
Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
            115                 120                 125

CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC        432
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
        130                 135                 140

AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGG        480
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

CAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC        528
His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC        576
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC        624
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG        672
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

AGC CTC TCC CTG TCT CCG GGT AAA TGA                                    699
Ser Leu Ser Leu Ser Pro Gly Lys *
225                 230
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1               5                  10                  15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
```

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Met Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145 150 155 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: FLAG® peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

CLAIMS

We claim:

1. A method of stimulating a humoral immune response, comprising administering a CD83 DNA and an antigen, in a pharmaceutically acceptable carrier.

2. The method according to claim 1, wherein the DNA is selected from the group consisting of:

(a) a DNA having a nucleotide sequence encoding an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2; and

(b) DNA molecules capable of hybridization to the DNA of (a) under stringent conditions and which encode biologically active CD83.

3. The method according to claim 1 wherein the DNA encodes the extracellular domain of CD83, which comprises amino acids 1 through 124 of SEQ ID NO:1.

4. The method according to claim 3, wherein the DNA is administered via intradermal injection.

5. A method of stimulating a humoral immune response, comprising administering a CD83 protein and an antigen, in a pharmaceutically acceptable carrier.

6. The method according to claim 5, wherein the CD83 protein is selected from the group consisting of:

(a) a CD83 peptide having an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2;

(b) fragments of a peptide according to (a) that have CD83 biological activity; and

(c) peptides encoded by DNA molecules capable of hybridization to a DNA encoding the peptide of (a) under stringent conditions, which are biologically active.

7. The method according to claim 6, wherein the CD83 protein comprises the extracellular domain of CD83.

8. The method according to claim 6, which further comprises administering a cytokine selected from the group consisting of Interleukins 1, 2, 4, 5, 6, 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a fusion protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon-γ; TNF; TGF-ß; flt-3 ligand; soluble CD40 ligand; biologically active derivatives of these cytokines; and combinations thereof.

9. A vaccine composition comprising a CD83 reagent selected from the group consisting of a DNA encoding CD83 and a CD83 protein, and an antigen, in a suitable diluent or carrier.

10. The vaccine composition according to claim 9, further comprising a cytokine selected from the group consisting of Interleukins 1, 2, 4, 5, 6, 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a fusion protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon-γ; TNF; TGF-ß; flt-3 ligand; soluble CD40 ligand; biologically active derivatives of these cytokines; and combinations thereof.

11. The vaccine composition according to claim 9, wherein the DNA encoding CD83 is selected from the group consisting of:

(a) a DNA having a nucleotide sequence encoding an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2; and

(b) DNA molecules capable of hybridization to the DNA of (a) under stringent conditions and which encode biologically active CD83.

12. The vaccine composition according to claim 10, wherein the DNA encoding CD83 is selected from the group consisting of:

(a) a DNA having a nucleotide sequence encoding an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2; and

(b) DNA molecules capable of hybridization to the DNA of (a) under stringent conditions and which encode biologically active CD83.

13. The vaccine composition according to claim 9, wherein the CD83 protein is selected from the group consisting of:

(a) a CD83 peptide having an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2;

(b) fragments of a peptide according to (a) that have CD83 biological activity; and

(c) peptides encoded by DNA molecules capable of hybridization to a DNA encoding the peptide of (a) under stringent conditions, and which encode biologically active CD83.

14. The vaccine composition according to claim 10, wherein the CD83 protein is selected from the group consisting of:

(a) a CD83 peptide having an amino acid sequence of amino acids 1 through 124 of SEQ ID NO: 2;

(b) fragments of a peptide according to (a) that have CD83 biological activity; and

(c) peptides encoded by DNA molecules capable of hybridization to a DNA encoding the peptide of (a) under stringent conditions, and which encode biologically active CD83.

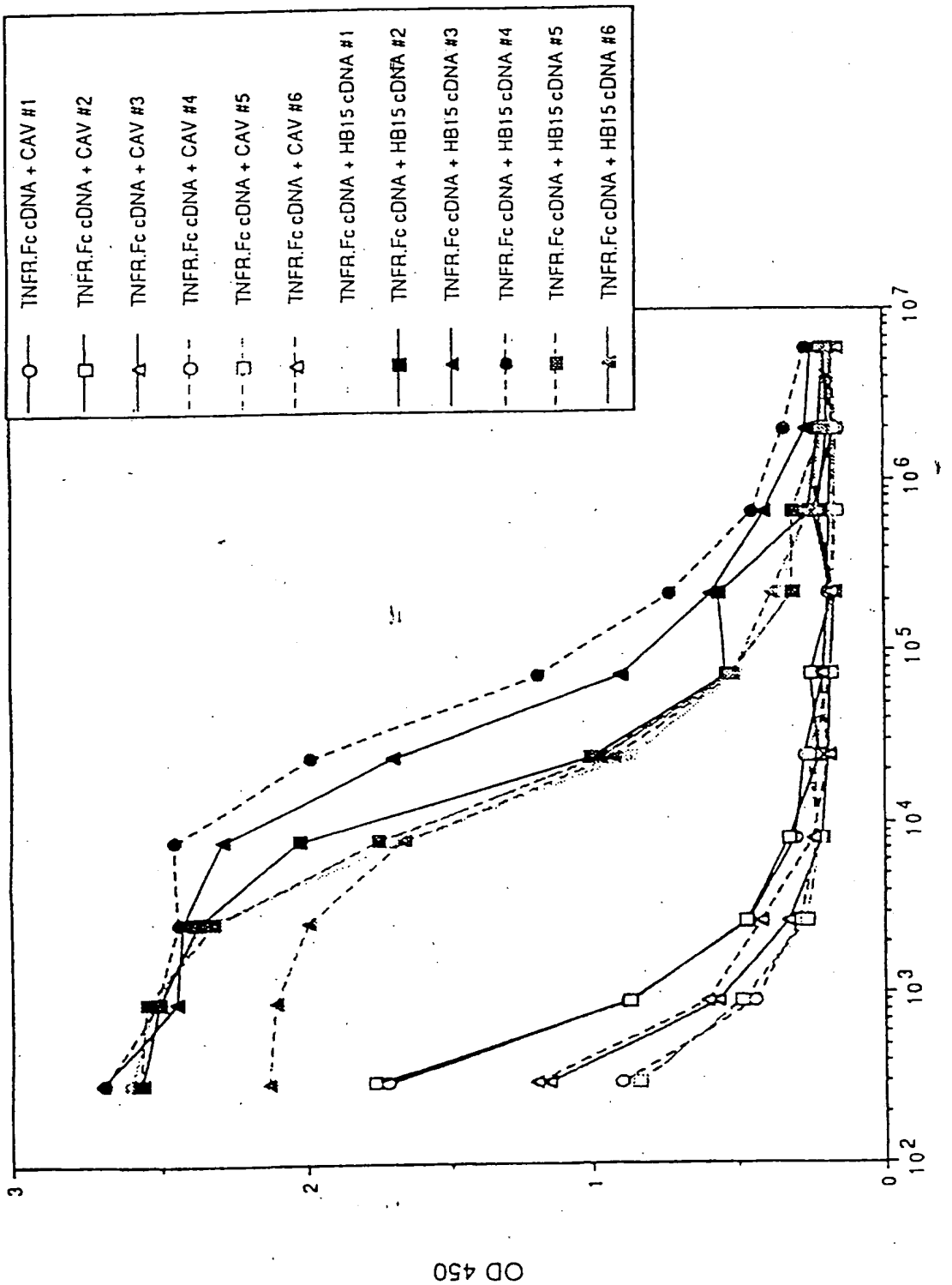
TNFR.Fc cDNA + CAV #1
TNFR.Fc cDNA + CAV #2
TNFR.Fc cDNA + CAV #3
TNFR.Fc cDNA + CAV #4
TNFR.Fc cDNA + CAV #5
TNFR.Fc cDNA + CAV #6
TNFR.Fc cDNA + HB15 cDNA #1
TNFR.Fc cDNA + HB15 cDNA #2
TNFR.Fc cDNA + HB15 cDNA #3
TNFR.Fc cDNA + HB15 cDNA #4
TNFR.Fc cDNA + HB15 cDNA #5
TNFR.Fc cDNA + HB15 cDNA #6
OD 450
0
1
2
3
10^2
10^3
10^4
10^5
10^6
10^7
Serum (reciprocal dilution)

FIGURE 1

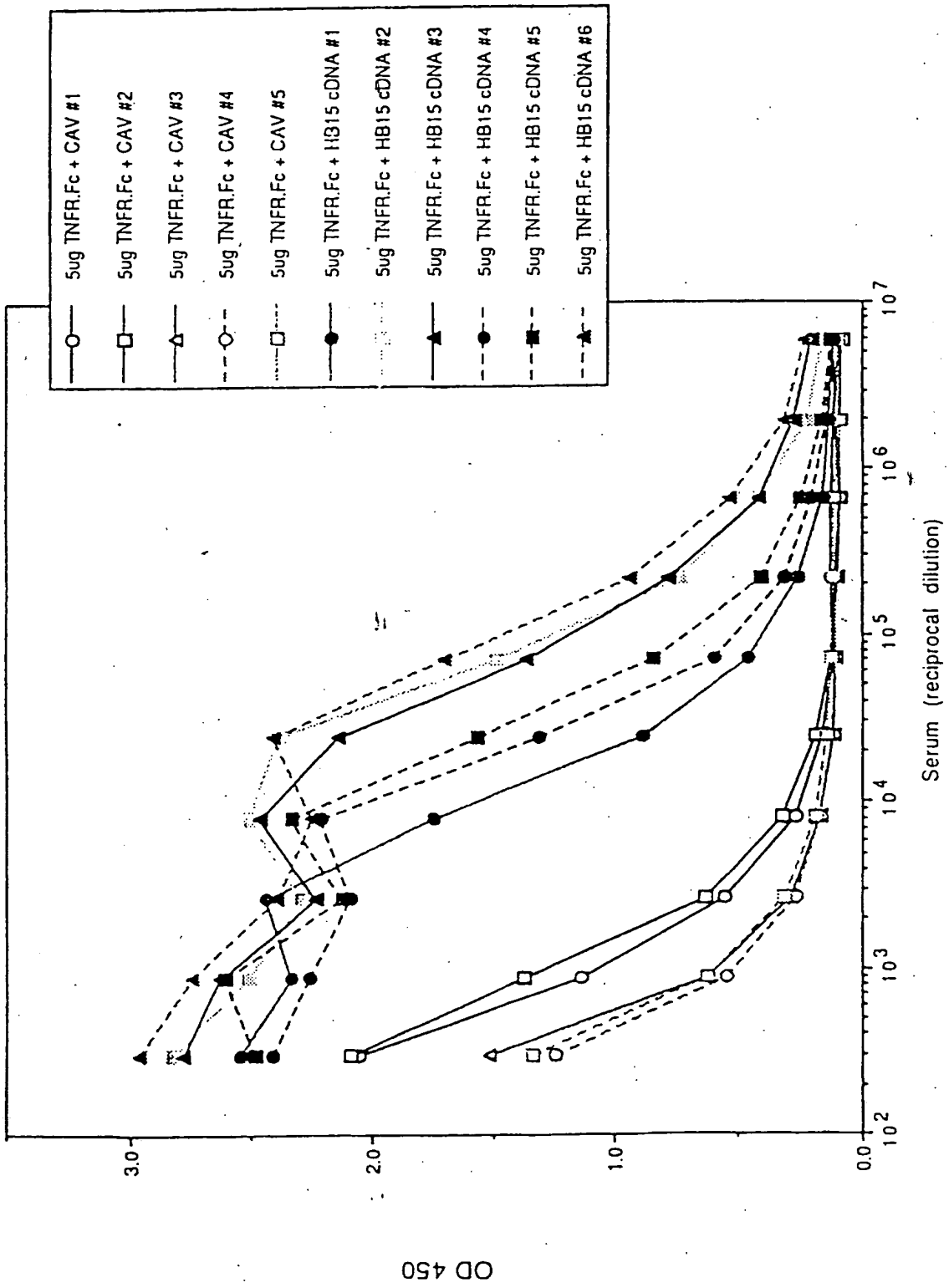
5ug TNFR.Fc + CAV #1
5ug TNFR.Fc + CAV #2
5ug TNFR.Fc + CAV #3
5ug TNFR.Fc + CAV #4
5ug TNFR.Fc + CAV #5
5ug TNFR.Fc + HB15 cDNA #1
5ug TNFR.Fc + HB15 cDNA #2
5ug TNFR.Fc + HB15 cDNA #3
5ug TNFR.Fc + HB15 cDNA #4
5ug TNFR.Fc + HB15 cDNA #5
5ug TNFR.Fc + HB15 cDNA #6
OD 450
0.0
1.0
2.0
3.0
10^2
10^3
10^4
10^5
10^6
10^7
Serum (reciprocal dilution)

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02350

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 48/00; C07K 5/00; C07H 21/04
US CL : 514/44; 530/300; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44; 530/300; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DIALOG, APS
search terms: CD83, HB15

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 93/21318 (DANA-FARBER CANCER INSTITUTE, INC.) 28 October 1993. See entire document.	1-7, 9, 11, 13 --- 8, 10, 12, 14
X -- Y	WO 95/29236 (DANA-FARBER CANCER INSTITUTE, INC.) 02 November 1995. See entire document.	9, 11, 13 --- 1-8, 10, 12, 14
Y	ENGEL et al. New CD from the B cell section of the fifth international workshop on human leukocyte differentiation antigens. Leuk. Lymphoma. 1994, Vol. 13, Suppl. 1, pages 61-64. See entire document.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 MARCH 1997

Date of mailing of the international search report
16 APR 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02350

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHOU et al. A novel cell-surface molecule expressed by human Interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily. The Journal of Immunology. 15 July 1992, Vol. 149, No. 2, pages 735-742. See entire document.	1-14
Y	ZHOU et al. A distinct pattern of cytokine gene expression by human CD83$^+$ blood dendritic cells. Blood. 01 November 1995, Vol. 86, No. 9, pages 3295-3301. See entire document.	1-14